The University of Chicago

THE EFFECT OF HIBERNATION AND SEASONAL VARIATION OF TEMPERATURE ON THE RESPIRATORY EXCHANGE OF *FORMICA ULKEI* EMERY

A DISSERTATION SUBMITTED TO THE
GRADUATE FACULTY IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY

By
WILLIAM ALBERT DREYER

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THE EFFECT OF HIBERNATION AND SEASONAL VARIATION OF TEMPERATURE ON THE RESPIRATORY EXCHANGE OF FORMICA ULKEI EMERY[1]

(Four figures)

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INTRODUCTION

HIBERNATION is a phenomenon of animal life which has long been recognized to be of fundamental importance and which has consequently attracted a large number of investigators. Aristotle observed hibernation in certain animals, both vertebrates and invertebrates. Gessner (1550) and Buffon (1749) recorded more extensive observations of the hibernating behavior of various animals. During the last century a large number of investigators have studied both the natural history and the physiological aspects of hibernation. A large amount of this work has been reviewed by Krogh (1916) and Holmquist (1925), so that it is unnecessary to repeat here. However, a study of some of the more outstanding works by Spallanzani (1803), M. Hall (1832), Barkow (1846), Valentin (1857–88), Schulz (1879), Pembrey (1898), Nagai (1909), Polimanti (1912), and Rasmussen (1916) gives us a conception of the significance and effects of hibernation. These observations were made principally upon hibernating mammals such as the marmot, hedgehog, bat, or dormouse, but also to some extent upon amphibians and reptiles and more rarely upon invertebrates including mollusks, crustaceans, and insects. The background furnished by these papers serves as an introduction to the present study and may be summarized briefly as follows:

In hibernation all organisms are more or less quiescent, both physically and physiologically. Heart rate is reduced from as much as several hundred beats per minute in small mammals to 10–20 beats

[1] This investigation was undertaken at the suggestion of Professor W. C. Allee, to whom the author wishes to express his appreciation for much valuable advice and friendly interest.

per minute; rate of respiration is reduced in similar proportion. Although some hibernating animals feed at intervals, all digestive and excretory processes are markedly reduced. Growth is greatly retarded or completely interrupted during hibernation, and accompanying this there is inhibition of sexual maturity and reproduction. Nervous sensitivity is greatly reduced. The body temperature indicates a decrease in heat regulation and varies within a few degrees of the environmental temperature. Even hibernating mammals lose effective temperature regulation, and the body temperature tends to approach that of the environment. In general, hibernation involves great reduction of all metabolic processes. Evidence of this is revealed in the greatly reduced gaseous exchange and the low respiratory quotient (R. Q.) which are recorded for the various animals studied.

As mentioned above, these observations were made primarily on mammals, reptiles, and amphibians. However, recognition of the phenomenon among invertebrates, especially insects, appeared as early as 1805, and interest in insect hibernation has continued steadily to date. Recently, Parhon (1909), Bodine (1923), Fink (1925*a*), and Holmquist (1928) have presented quantitative data on the physiological effects of hibernation on bees, grasshoppers, a potato beetle, and an ant, respectively. In the work on ants Holmquist used *Formica ulkei*, so that some preliminary data on respiration in his 1928 paper serve as a point of departure for the present investigation. It has been found that the general physiological effects of hibernation on these insects are quite similar to those described for mammals and other vertebrates, i.e., a condition of greatly reduced metabolic activity occurs.

In addition to these studies of hibernation much attention has been directed during the last thirty years to the study of insect physiology in general. The effects of various environmental factors on rate of growth, rate of reproduction, and particularly on respiratory exchange, have been observed. The latter has been especially emphasized in studying the physiological condition of animals, inasmuch as the gaseous exchange and the R. Q. are interpreted as direct indicators of the intensity and nature of the metabolic processes. The large amount of literature in this field has been reviewed

by Battelli and Stern (1913) and more recently by Sayle (1928), and will be referred to in greater detail only in conjunction with the experimental results.

In these physiological studies of insects, temperature is one of the salient factors taken into account. Similarly, in any physiological study of hibernation, temperature is one of the significant factors to be considered. Especially does this appear to be true when one observes that hibernation occurs among animals in which the body temperature is entirely dependent upon the environmental temperature, i.e., among poikilothermal animals and among homoiothermal animals in which the heat-regulating mechanism is inefficient or fails to function during hibernation. Since temperature is such an important factor in the physiology of hibernation, it is the purpose of this paper to analyze the respiratory exchange of the mound-building ant, *Formica ulkei* Emery, in respect to seasonal temperature changes and its hibernating behavior. In its prosecution the work has been subdivided as follows:

1. What is the normal respiratory exchange of *Formica ulkei* during the different seasons of the year?

2. What is the effect of constant activity or constant hibernation throughout the four seasons on its respiratory behavior?

3. Are the normal seasonal changes in respiratory exchange directly correlated with temperature, or is there an inherent seasonal rhythm?

4. What are the effects of differential feeding and starvation on *Formica ulkei?*

5. What is the relation of such effects to the respiratory phenomena observed in hibernating ants?

MATERIAL AND METHODS

The experimental animals were maintained in the laboratory in large paraffin-lined galvanized iron tubs in which all natural conditions of temperature, humidity, soil moisture, food, etc., were carefully imitated. The various experimental conditions were provided in rooms of controlled temperature and humidity. Under such conditions the ants constructed typical large mounds and exhibited behavior normal for the given temperature. The ants that were kept

active at 22° C. indulged in their characteristic practice of trophallaxis, and normal reproduction took place with several queens present in each colony. Those that were kept at 4° C. remained inactive and aggregated in the galleries as in hibernation in nature. The respiratory behavior of the laboratory colonies was found to be similar to that of animals brought directly from the field nests. This imitation of natural conditions is important in all stocks kept for physiological experimentation, and should be given more consideration than it has received in many earlier investigations.

The method adopted for the measurement of the gaseous exchange of the ants is a micro-respirometer method originally described by Harrington and Crocker (1923). A modification of the animal chamber of this apparatus for use with the ants was described by Holmquist (1928). Several further modifications have been adopted which must be mentioned here. The mercury manometer was replaced by a kerosene manometer made of smaller bore tubing, about 1.5 millimeters in diameter, in order to increase the sensitivity of the apparatus. A correction was introduced into the calculation to deduct the volume of CO_2 normally present in the atmosphere (.03–.04 per cent). A molar solution of KOH was used for the absorption of carbon dioxide. The remainder of the use of this method was according to the directions given by the authors. The volumes of the gases were calculated according to the formula

$$V_1 = F \times V \times P ,$$

in which V_1 is the volume of the gas to be calculated, V is the volume of the apparatus minus the volume of experimental material, P is the barometric pressure, and F is a factor for conversion to standard conditions. The principal advantage of this type of respirometer is that it permits the simultaneous determination of both carbon dioxide and oxygen. Consequently, the significance of differences in the gaseous exchange can be indicated by the changes in the value of the respiratory quotient (R. Q. $= CO_2/O_2$). In addition, it is a convenient piece of apparatus, easy to build and repair and easy to operate.

A uniform type of experiment measuring the respiration of 25 workers for 105 minutes at a constant temperature, as indicated in the tables, was used throughout. This number of animals and length

of time was found to provide optimum normal conditions of activity and crowding of the ants.[1] Experiments were conducted at 4° C. on hibernating animals and at 22° C. on active animals. These temperatures were selected to correspond to average winter and summer temperatures for the ant mounds (Holmquist, 1928). During spring and fall, experiments were conducted at several intermediate temperatures—7°, 10.5°, 15.5°, and 18.5° C.—as shown by the tables. For the sake of compactness in the tables, the figures given do not represent single experiments but are in each case an average of from three to six individual experiments performed on and near the date given. All volumes of carbon dioxide and oxygen are given in cubic millimeters and all temperatures in degrees Centigrade.

EXPERIMENTAL RESULTS

1. SEASONAL RESPIRATORY EXCHANGE

In order to properly evaluate the effect of temperature upon the respiratory behavior of *Formica ulkei*, it was first necessary to have an accurate record of the normal seasonal gaseous exchange. Experiments measuring the gaseous exchange of ants taken from a colony maintained outdoors at the laboratory were performed at intervals of 2 to 3 weeks from February, 1930, to April, 1931. Table I summarizes these results, as described above. The accompanying graph, Figure 1, clearly shows the seasonal fluctuations which occur in gaseous exchange and the close correlation which they bear to the temperature at which the exchange was measured. In the winter during hibernation the gaseous exchange was low, 55 cu. mm. of CO_2 to 110 cu. mm. of O_2, with an R. Q. of about .500. As the temperature rose during the spring, the respiratory rate increased, with an attendant rise in R. Q. In the summer the gaseous exchange was high—for instance, 277 cu. mm. of CO_2 to 314 cu. mm. of O_2 with an R. Q. of .882. As the temperature fell in the autumn, the respiratory

[1] The ants were collected from the nests by allowing them to crawl on strips of filter paper, which were then dropped into large flasks. By cooling the flasks for 15 minutes, the ants were rendered inactive long enough to place 25 of them in one of the animal chambers. Another 15 minutes was allowed for the animal chamber and ants to return to room temperature. Such handling was necessary to prevent abnormal disturbance of the ants, which is always accompanied by the exudation of formic acid, the fumes of which are fatal to the ants in a closed space.

exchange and the R. Q. declined until hibernating conditions were restored.[1] Similar experiments were performed seasonally on ants brought directly from field nests (Table I), and the same seasonal

TABLE I

RESPIRATORY EXCHANGE—SEASONAL—1930–31

(25 Animals—105 Minutes)

LABORATORY CONTROL ANTS					FIELD ANTS				
Date	Temp. (Degrees Cent.)	Cmm. CO_2	Cmm. O_2	R. Q.	Date	Temp. (Degrees Cent.)	Cmm. CO_2	Cmm. O_2	R. Q.
2/6/30	4	55	110	0.500	2/4/30	4	56	111	0.504
2/25	4	52	105	.495					
3/3	6	54	85	.636					
4/15	10.5	101	151	.668					
5/13	15.5	128	180	.711					
5/27	18.5	176	225	.782					
6/6	22	228	260	.876	4/30	22	236	270	.874
6/27	22	254	296	.858					
7/12	22	262	306	.856					
8/12	22	277	314	.882	8/13	22	279	320	.871
8/20	22	218	243	.897					
9/25	22	265	303	.874					
10/20	18.5	165	208	.796	10/17	18.5	165	211	.782
10/27	15.5	141	201	.701					
11/9	10.5	112	174	.643					
12/1	7	69	121	.570					
1/12/31	4	55	107	.514	11/25	4	53	104	.509
1/30	4	57	119	.479					
2/2	10.5	55	110	.500*	2/6	10.5	64	117	.547*
3/10	4	55	107	.514	2/9	4	55	108	0.509
4/14	10.5	104	161	.644					
4/25	15.5	123	173	0.711					

* Compare with entry for 2/2 and 10.5° in Table VIII.

fluctuation was observed. The close correlation of the two sets of data in conjunction with the observation of normal activity and behavior indicate that this is the typical annual cycle for *Formica ulkei*.

2. RESPIRATORY EXCHANGE AT CONSTANT TEMPERATURES

Parallel series of experiments conducted with animals taken from colonies maintained at constant temperatures throughout the year

[1] The temperature at which the experiments were conducted, as indicated in the tables, are more significant than the dates, which merely indicate the seasonal sequence. In spring and fall certain temperatures between 4° and 22° C. were selected (Tables I, VII, and VIII) at which experiments were conducted to show the progress of exit from and entrance to hibernation.

showed that under such conditions the respiratory behavior remains unchanged (Table II and Fig. 2).[1] When held at a constant temperature of 22° C., the ants maintained the high rate of respiration normal for summer throughout the winter, with an R. Q. between

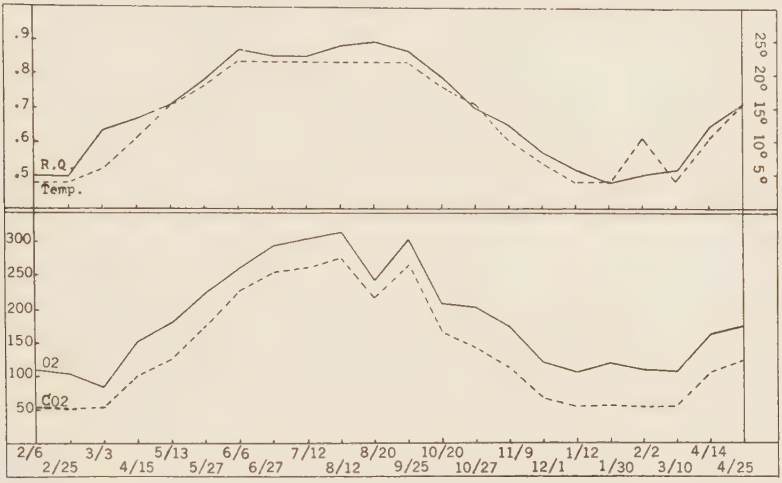

FIG. 1.—Graph for Table I showing seasonal gaseous exchange of ants. Ordinates are cubic millimeters of gas in lower half, and R. Q. on left and temperature on right in upper half. Abscissas are dates of experiments. Solid line is O_2 below, R. Q. above. Broken line is CO_2 below, temperature of the experiment above.

.800 and .900. The fact that the ants not only maintained their activity all winter but also reproduced normally in February and March is interpreted as additional evidence that their physiological condition during the winter, when subjected to the high temperature, was the same as it is normally during the summer. On the other hand, when a constantly low temperature was maintained, the ants continued to hibernate all summer, with a respiratory rate and R. Q.

[1] When these data were analyzed according to Student's Method for comparing two sets of unpaired experiments, the difference between the CO_2 production of hibernating and active ants was found to be highly significant statistically. The calculation gave a value of $t = 7.24$, which is equivalent to a probability of less than .00000001. The difference between the O_2 consumption of hibernating and active animals and the differences in gaseous exchange between normal and starved animals shown later are of similar magnitude, so that no further statistical analysis was deemed necessary.

similar to those observed normally in the winter.[1] There was no seasonal effect on either the volume or the character of the respiratory exchange when the ants were kept at either a constant high or a constant low temperature.

TABLE II

RESPIRATORY EXCHANGE—CONSTANT TEMPERATURE

(25 Animals—105 Minutes—22° and 4° C.)

CONSTANT ACTIVITY—22° C.				CONSTANT HIBERNATION—4° C.			
Date	Cmm. CO_2	Cmm. O_2	R. Q.	Date	Cmm. CO_2	Cmm. O_2	R. Q.
12/8/29	244	280	0.871	12/6/29	55	110	0.500
1/24/30	249	287	.867	1/20/30	69	136	.507
2/10	187	223	.838	1/31	41	76	.540
3/10	212	241	.879	2/23	45	89	.505
4/25	241	271	.889	3/6	48	95	.505
5/1	262	323	.842	4/3	43	89	.483
6/10	266	294	.904	4/29	57	111	.513
6/18	219	242	.904	5/18	51	97	.525
7/2	233	271	.859	6/22	53	107	.495
7/10	262	317	.826	7/7	48	91	.527
7/23	257	293	.877	7/24	47	98	.479
8/12	279	320	.871	8/14	50	99	.505
8/22	271	299	.906	8/22	56	110	.509
10/22	236	275	.858	9/20	49	105	.466
10/30	256	303	.844	10/20	47	97	.484
11/13	270	331	.815	10/31	58	119	.487
12/5	287	333	.861	11/14	56	112	.500
12/23	254	287	.885	11/25	53	104	.509
1/8/31	244	275	.887	12/23	61	119	.512
1/20	201	231	.870	1/13/31	58	112	.517
1/29	242	271	.895	1/28	47	96	.489
2/9	239	284	.841	2/9	56	112	.500
4/14	271	304	0.891	4/15	50	100	0.500
Mean.....	247	285	0.866	Mean.....	52	104	0.500

Table III shows the mean volumes of gaseous exchange and R. Q. obtained for the various experimental temperatures. It is evident that the volume of gaseous exchange and the R. Q. increase directly with the temperature. The reduction in volume of gaseous exchange observed during hibernation amounts to 79 per cent in the case of the CO_2 and 63 per cent for the O_2. Inasmuch as the CO_2 is re-

[1] Somewhat greater variation occurred in the volumes of gaseous exchange observed at 22° C. than at 4° C., owing to the fact that spontaneous activity is much more variable in the case of the active ants.

duced more than the O_2, there follows the lowering of the R. Q. from a mean of .866 at 22° C. to a mean of .500 at 4° C. The right-hand division of Table III gives the gaseous exchange in cu. mm. of gas

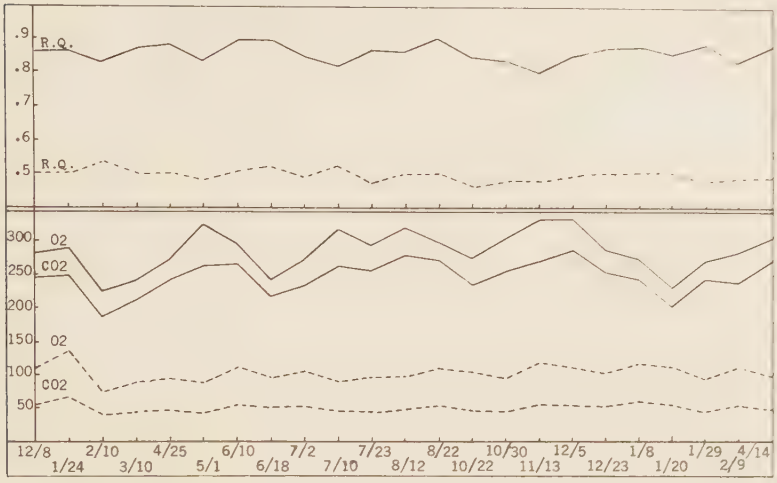

FIG. 2.—Graph for Table II showing gaseous exchange of ants at constant temperature. Ordinates are cubic millimeters of gas in lower half, and R. Q. in upper half. Abscissas are dates of experiments. Solid lines indicate experiments at 22° C. Broken lines indicate experiments at 4° C.

TABLE III

STANDARD RESPIRATORY EXCHANGE

(Cubic Millimeters per Gram per Hour)

TEMP. (DEGREES CENT.)	CMM. PER .192 GR. PER 105 MIN.			CMM. PER GR. PER HR.		
	Cmm. CO_2	Cmm. O_2	R. Q.	Cmm. CO_2	Cmm. O_2	R. Q.
4.0.....	52	104	0.500	154.75	309.50	0.500
7.0.....	62	103	.601	184.51	306.53	.601
10.5.....	104	162	.641	309.50	482.11	.641
15.5.....	135	191	.706	401.76	568.42	.706
18.5.....	171	217	.788	508.89	645.79	.788
22.0.....	247	285	0.866	735.07	848.16	0.866

per gram body weight per hour to aid in the comparison with the results of other workers which are similarly given. The experimental results presented thus far afford some interesting comparisons with those of numerous investigators working with different insects.

3. RESPIRATORY EXCHANGE OF OTHER INSECTS

In a similar seasonal study of the respiratory exchange of bees, Parhon (1909) found that a relatively constant oxygen consumption of about 20,000 cu. mm. per gram per hour took place throughout the year. Further, the greatest volume of gaseous exchange was at 10° C., so that the curve of gaseous exchange for the bees at various temperatures from 0° to 32° C. resembles that of a homoiothermal animal. Parhon attributed this phenomenon and the seasonal constancy to the social heat regulation of the bee colony by which the hive temperature is normally kept at 30°–32° C. throughout the year. Obviously, if temperature is an important factor affecting the respiratory rate, such a result is to be expected in contrast to the fluctuating seasonal exchange of the ants which are living in the mounds with the temperature at a mean of 22° C. in the summer and 4° C. in the winter (Holmquist, 1928). In contrast to the constant exchange of the bees, Parhon found that the O_2 consumption of flies increased with the temperature from 0 cu. mm. per gram per hour at 10° C. to 14,000 cu. mm. at 37° C. In all experiments on both bees and flies, the CO_2 production was very nearly equal to the O_2 consumption, so that the R. Q. fluctuated around 1.0.

Vansell (1929) observed a relatively constant CO_2 production of 13,000–15,000 cu. mm. per gram per hour for bees in summer and winter. Battelli and Stern (1913) reported that flies at 20° C. produced 2,300 cu. mm. per gram per hour of CO_2 and consumed 3,100 cu. mm. of O_2. It is to be noted that these are very high rates of respiratory exchange. Parhon, in a comparative table, pointed out that the rate for the bees greatly exceeds that of birds, various mammals, reptiles, and other animals. In general it is assumed that the respiratory exchange of insects is relatively greater than that of other animals. However, the volumes of gaseous exchange reported here for *Formica ulkei* and the results for other insects summarized below are quite comparable to the volumes given for some of the small mammals and birds, i.e., 700–1,000 cu. mm. per gram per hour. The volumes observed for several different nymphs and pupae are even lower, ranging from 350 to 500 cu. mm. per gram per hour.

Other investigators, such as Rogers (1929) and Bodine (1923), working with grasshopper nymphs; Sayle (1928), with dragon-fly

nymphs; Battelli and Stern (1913), with May beetles, silkworm moths, and flies; and Slowtzoff (1909), with manure beetles and ants, found that the volume of gaseous exchange and the R. Q. increased with the temperature and that the volumes between 0° and 5° C. were 75–95 per cent less than those measured at 20–25° C. Thus, some insects show an even greater reduction at low temperatures than that reported for *Formica ulkei*, although none of these insects show as great a reduction as that observed in the case of some hibernating mammals. Regnault and Reiset have reported that the respiration of the hibernating marmot is reduced thirty times.

Tauchert (1929) reported that mealworms, May beetles, and *Vanessa* normally consume as much as 800 cu. mm. per gram per hour of O_2. Fink (1925*a*) found that the potato beetle, when active, produces 714 cu. mm. per gram per hour of CO_2. This volume was reduced 70–80 per cent during hibernation. The R. Q. of the potato beetle also was reduced from .76 to .60 during the early stages of hibernation, although it rose again during the later stages. Frew (1929) mentioned unpublished data which indicate that the R. Q. of insects was reduced from .72–.80 during activity to .41–.71 during hibernation. Bodine (1923), studying hibernation of grasshopper nymphs, reported a CO_2 production of 398 cu. mm. per gram per hour at 18°–20° C. This was reduced to $\frac{1}{8}$–$\frac{1}{10}$ of that amount between 0° and 8° C. Regen (1906) obtained a similar reduction in the CO_2 production of a cricket. In another case, Bodine (1921*a*) found that the CO_2 output of grasshopper nymphs was 637 cu. mm. per gram per hour at 20° C. and 255 cu. mm. at 5° C., a decrease of 60 per cent. Battelli and Stern (1913) observed that silkworm moths produced 900 cu. mm. per gram per hour of CO_2 at 20° C. and that these volumes increased at 30° and 40° C. The R. Q. also increased from .64 to .69. Similar results were obtained with May beetles, which produced 610 cu. mm. per gram per hour of CO_2 at 20° C. with an R. Q. of .65. The R. Q. for the flies mentioned above was .74 at 20° C.

Slowtzoff (1909) found that the CO_2 production of manure beetles and ants increased with temperature amounting to about 400 cu. mm. per gram per hour of CO_2 at 20° C. Such a volume is somewhat lower than that reported for *Formica ulkei* and for other beetles, and

may be associated with a variation in the response of the respiratory exchange to temperature which was pointed out by Slowtzoff. He observed a temperature interval of about 12° beginning at different levels (i.e., 15°–25° C.) in different insects within which no increase in respiratory rate occurred. Similar observations were made by Vernon (1897), Schulz (1879), and Bütschli (1874). No such static period was observed in the respiratory behavior of *Formica ulkei* or in the observations on other insects described above, so that considerably higher rates of gaseous exchange were recorded for experiments at 20°–25° C., when the increase in rate extended over the whole temperature range. Slowtzoff reported an R. Q. of .8 for the beetles, .9 for the ants, and 1.0 for the flies. Regnault and Reiset, as early as 1849, found that the cockroach consumed 727 cu. mm. of O_2 per gram per hour and that the May beetle used 716 cu. mm. of O_2 with an R. Q. of .8. In general the results of these different investigators, who used a variety of insects, exhibit considerable uniformity to which the present data ascribe. With such a basis for comparison, further experimental results can now be described.

4. EFFECT OF TEMPERATURE REVERSAL ON RESPIRATORY EXCHANGE

In order to further test the effect of temperature independently of the seasons, ants were taken from the constant 22° colonies and subjected to a temperature of 4° C. for 1–2 hours. When the respiration was then measured at 4° C., the volume of gaseous exchange was reduced as it is normally during hibernation. Similarly, the R. Q. was reduced to about .500. Subsequently, when the same ants were restored to a temperature of 22° C. for 1–2 hours, they regained their normal activity, and experiments conducted at 22° C. showed a recovery of the normal rate and R. Q. for that temperature (Table IV). Conversely, when ants from the colonies subjected to constant hibernation were warmed to 22° C. and the respiration was measured, the same type of reversal occurred. In this case the volume of gaseous exchange increased, and the R. Q. was raised to .800 to .900. Evidently the respiratory rate and the R. Q. are functions of the experimental or environmental temperature. Kayser and Gingling-er (1927), experimenting on non-hibernating animals such as the tortoise, mouse, rabbit, pigeon, and guinea pig, have expressed this

same opinion. They observed an increase in R. Q. with increase in experimental temperature and interpreted the R. Q. as a function of temperature, considering it as a factor independent of muscular activity or digestive processes. The R. Q. obtained represents the algebraic sum of the R. Q. of each of the several oxidative processes going on at any one time. The equilibrium of these oxidative processes varies with temperature just as it does with change in concen-

TABLE IV

RESPIRATORY EXCHANGE—TEMPERATURE REVERSAL

(25 Animals—105 Minutes)

NORMAL ANTS				HIBERNATING ANTS			
	Cmm. CO_2	Cmm. O_2	R. Q.		Cmm. CO_2	Cmm. O_2	R. Q.
Exp. at 22° C.	244	275	0.887	Exp. at 4° C.	50	98	0.510
Exps. at 4° C.	42	75	0.560	Exps. at 22° C.	195	203	0.960
	57	105	.542		280	317	0.883
	37	66	.616		196	194	1.01
	19	37	.513		203	260	0.780
	37	54	.685		256	318	.805
	32	56	.571		225	274	.821
	46	107	.430		201	215	.934
	59	147	.401		206	221	.932
	35	80	.430		166	182	.912
	58	96	.604		213	248	.858
	51	126	0.404		193	201	0.960
Mean.....	43	86	0.498	Mean.....	212	239	0.886
Same at 22° C.	226	258	0.876	Same at 4° C.	54	103	0.524

tration of the cellular elements. Consequently, the R. Q. varies with the oxidative processes according to temperature. Such facts seem quite in accord with the present data and thoroughly acceptable in view of the current physiological interpretations of the R. Q. as an indicator of the character of metabolic processes.

5. EFFECT OF DIFFERENTIAL FEEDING ON RESPIRATORY EXCHANGE

A large number of investigators have shown that the R. Q. of mammals varies with the diet in such a manner that the R. Q. for a carbohydrate diet is approximately 1.0; for protein, .80; for fat, .70 (Krogh, 1916). When three like colonies of *Formica ulkei* kept at

22° C. were subjected to such differential feeding, the R. Q. was similarly observed to be an indicator of the character of the metabolism (Table V). The experiments on the carbohydrate-fed ants showed an R. Q. fluctuating around 1.0 with a mean of .984. Parhon (1909) found that bees (which were living on a carbohydrate diet) also exhibited an R. Q. of about 1.0. In the case of the ant colonies fed on fat and on protein, the R. Q. in the beginning, .63 and .71, respectively, was indicative of the diet; but subsequently the effect

TABLE V

RESPIRATORY EXCHANGE—DIFFERENTIAL FEEDING*

(25 Animals—105 Minutes—22° C.)

DATE	CARBOHYDRATE†			FAT			PROTEIN		
	Cmm. CO_2	Cmm. O_2	R. Q.	Cmm. CO_2	Cmm. O_2	R. Q.	Cmm. CO_2	Cmm. O_2	R. Q.
7/24/30	290	314	0.923	175	276	0.634	138	194	0.711
8/1	243	231	1.05	133	223	.596	80	155	.516
8/6	260	278	0.935	94	191	.492	106	157	.675
8/19	252	235	1.07	91	152	.598	46	95	.484
8/26	243	250	0.972	55	106	0.518	42	85	0.494
Mean..	258	262	0.984	110	190	0.579	82	137	0.600

* These colonies were starved 2 weeks and then fed the indicated diet for 1 week before these experiments were begun.

† The R. Q. expected from a carbohydrate diet is 1.0; from fat, .70; from protein, .80.

of starvation was superimposed on that of the limited diet and both the volume of the gaseous exchange and the R. Q. were further reduced.[1] As in the case of the effect of temperature, the respiratory rate and the R. Q. are indicators of the intensity and character of the metabolic activity of the ants. Under these conditions they are evidently functions of the food supply. Other investigators, namely, Slowtzoff (1909) and Gourévitch (1928), have come to this same conclusion. Slowtzoff found that the R. Q. for flies fed on sugar was 1.0. When they were fed on meat for several days, the quotient fell to .8. The latter author found that the R. Q. of the cockroach was .65–.84 after several days of starvation; .78–.83 when fed on fresh cream; and 1.01–1.07 when fed on starch.

[1] See section 6, on the effects of starvation on the volume of gaseous exchange and the R. Q.

6. EFFECT OF STARVATION ON RESPIRATORY EXCHANGE

Along with the three colonies fed on the restricted diets, a fourth colony, also kept at 22° C., was subjected to starvation. In Table VI and Figure 3, the data obtained from experiments at 22° C. on normal and starving ants are compared. It is evident that starvation causes a progressive reduction in the volume of gaseous exchange accompanied by a reduction of the R. Q. from the normal level, .800–.900, to .450, indicating a reduction in metabolic activity and an alteration in its character comparable to that observed generally in mammals, including man. After 5 weeks of starvation, the CO_2 production was reduced 72 per cent, while after 8 weeks it was reduced 92 per cent. At the same intervals the O_2 consumption was reduced 66 per cent and 85 per cent, respectively. Thus the reduction in metabolic activity produced by starvation is considerably greater than that produced by low temperature during hibernation.

TABLE VI

RESPIRATORY EXCHANGE—STARVED ANTS

(25 Animals—105 Minutes—22° C.)

DATE	NORMAL ANTS			STARVED ANTS		
	Cmm. CO_2	Cmm. O_2	R. Q.	Cmm. CO_2	Cmm. O_2	R. Q.
7/2/30	233	271	0.859	213	255	0.835
7/10	265	319	.830	190	267	.711
7/16	259	295	.877	156	246	.634
7/24	268	306	.875	93	157	.592
7/31	243	275	.883	75	131	.572
8/6	280	313	.894	60	112	.535
8/19	286	317	.902	56	123	.455
8/26	256	280	0.914	18	40	0.450

In this connection Tauchert (1929) observed that the O_2 consumption of meal worms, May beetles, and *Vanessa*, when fasting, was lowered 50–75 per cent. Sayle (1928) reported that the CO_2 production of dragon-fly nymphs was decreased 50 per cent during 4 weeks of starvation. Davis and Slater (1926) believe that starvation depresses the R. Q., for they found that cockroaches have an R. Q. between .72 and 1.0 when fed but that the R. Q. was between .75 and .85 when the animals were starved. Fink (1925*a*) found that a month

of starvation reduced the CO_2 production of the potato beetle from 714 to 153 cu. mm. per gram per hour, about 79 per cent. Bodine (1921*a*) reported that 120 hours of starvation caused a decrease in the CO_2 production of certain adult grasshoppers from 497 to 363 cu.

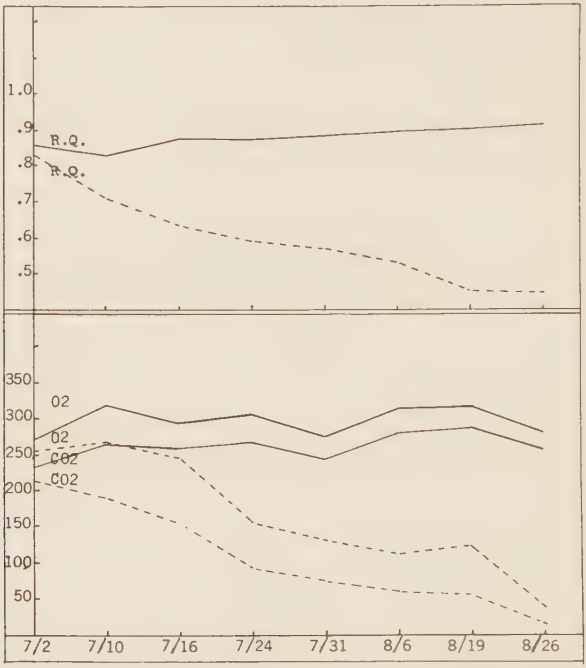

FIG. 3.—Graph for Table VI showing gaseous exchange of ants during 8 weeks of starvation at 22° C. Ordinates are cubic millimeters of gas below, R. Q. above. Abscissas are dates of experiments. Solid lines indicate experiments on normal ants. Broken lines indicate experiments on starving ants.

mm. per gram per hour, a decrease of about 27 per cent. Other experiments showed a reduction up to as much as 54 per cent. Thus the effect of starvation on the gaseous exchange of *Formica ulkei* is similar to its effect upon the metabolism of numerous other insects.

7. EFFECT OF STARVATION UPON RESPIRATORY EXCHANGE DURING HIBERNATION

During the course of the experiments certain relations between the effects of starvation and hibernation were observed. In the left-

hand division of Table VII and in Figure 4 are recounted the results of experiments on the respiration of ants during the normal exit from hibernation in the spring. Under these conditions the rate of gaseous exchange and the R. Q. increased with the rising temperature until they reached the level shown to be typical of the active ants in summer at a constant temperature of 22° C. At 10.5° C. some of the ants were segregated so as to be unable to begin feeding, which they do normally as activity increases above that temperature. Although

TABLE VII

RESPIRATORY EXCHANGE—EFFECT OF STARVATION UPON EXIT FROM HIBERNATION

(25 Animals—105 Minutes)

CONTROL-NORMAL EXIT					UNFED-ABNORMAL EXIT				
Date	Temp. (Degrees Cent.)	Cmm. CO_2	Cmm. O_2	R. Q.	Date	Temp. (Degrees Cent.)	Cmm. CO_2	Cmm. O_2	R. Q.
2/25/30	4	52	105	0.495	2/25/30	4	52	105	0.495
3/3	6	54	85	.636	3/3	6	54	85	.636
4/15	10.5	101	151	.668	4/15	10.5	101	151	.668
5/13	15.5	128	180	.711	5/8	15.5	92	161	.571
5/27	18.5	176	225	.782	5/17	18.5	94	150	.626
6/6	22	228	260	.876	5/27	22	111	185	.600
6/27	22	254	296	.858	6/8	22	221	254	.870*
7/12	22	262	306	0.856	6/17	22	157	243	0.646†

* Same ants as 5/27 after feeding 10 days.

† Same ants as 6/7 after starving again for 10 days.

the rising temperature stimulated activity, the volume of gaseous exchange and the R. Q. were reduced by the attendant starvation and remained far below the normal for the temperatures at which the experiments were conducted (right-hand division of Table VII). Later, when these ants, now at 22° C., were supplied with food, they recovered the normal respiratory behavior (entry for June 8, Table VII). Finally, subsequent starvation incited a secondary decline, as shown by the entry for June 17.

The same sort of effect of starvation was observed during the winter of 1931, when unusually warm weather raised the temperature of the mounds, both outdoors at the laboratory and in nature, to 10°–12° C. This higher mound temperature was accompanied by

an increase in the activity, and consequently in the metabolic rate, of the ants in hibernation. However, no feeding took place, and experiments performed at 10.5° C. (Table VIII) did not show the expected increase in the respiratory activity or R. Q. The latter re-

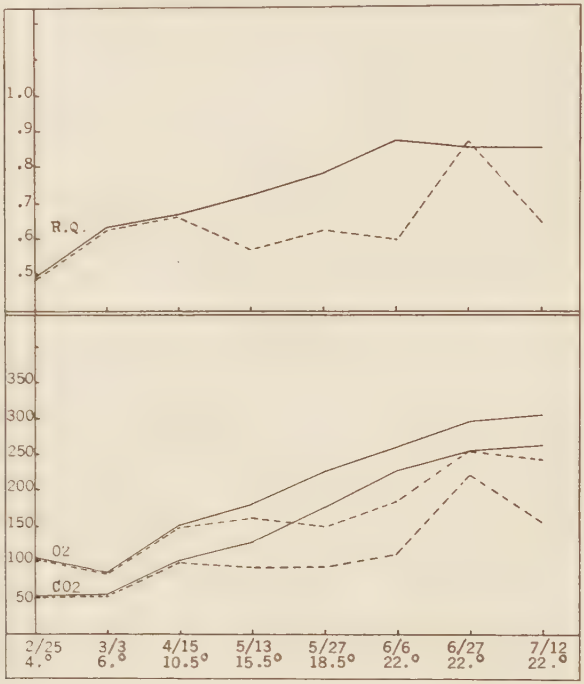

FIG. 4.—Graph for Table VII showing gaseous exchange of ants during exit from hibernation. Ordinates are cubic millimeters of gas below, R. Q. above. Abscissas are dates and temperatures of experiments. Solid lines indicate experiments during normal exit. Broken lines indicate experiments during abnormal, starved exit.

mained around .500, as in the case of ants normally hibernating at 4° C. Further, when these ants were warmed to 22° C., the gaseous exchange and the R. Q. were still below normal, as in the case of the ants starved during the exit from hibernation in the spring (Table VII). Experiments performed on ants brought directly from the field yielded analogous results (right-hand division of Table VIII). However, when these ants were fed and subsequently tested, the results showed a recovery of the respiratory behavior shown to be typical

of 10.5° C. (entry for February 24, Table VIII). Later in the winter when cold weather had restored the mound temperature to the normal level, experiments, conducted as usually at 4° C., revealed the characteristic low rate of respiration with an R. Q. about .500, which was shown to be typical of hibernation. Finally, some experiments performed at 10.5° C. on ants taken at this time from the colony subjected to constant hibernating conditions showed a normal increase in respiration in conjunction with the increase in temperature. Ap-

TABLE VIII

RESPIRATORY EXCHANGE—EFFECT OF STARVATION DURING HIBERNATION

(25 Animals—105 Minutes)

LABORATORY CONTROL ANTS					FIELD ANTS				
Date	Temp. (Degrees Cent.)	Cmm. CO_2	Cmm. O_2	R. Q.	Date	Temp. (Degrees Cent.)	Cmm. CO_2	Cmm. O_2	R. Q.
1/12/31	4	55	107	0.514	2/9/31	4	55	108	0.509
1/30	4	57	119	.479	2/6	10.5	64	117	.547
2/2	10.5	55	110	.500	2/23	22	124	189	0.656
2/3	22	152	236	.644					
2/24	10.5	93	153	.607*					
3/10	4	55	107	0.514					
Normal means for	4	51	100	0.505					
each temperature	10.5	98	160	.612					
	22	239	273	0.875					

* Same ants as 2/2 after feeding 3 weeks.

parently the increased rate of metabolism during the warm weather caused the ants to undergo some starvation in the absence of food. Normally the greatly reduced metabolism accompanying hibernation protects them from such starvation. Marked variations occur in this respect in different animals. For instance, Bodine (1923) pointed out that hibernating grasshopper nymphs become active at intervals and feed and that starved individuals do not survive the period of hibernation. The torpidity of such insects in hibernation is not so great as in the case of the ants, so that their need for food is due in part to the fact that metabolic activity is not reduced to a minimum, as is that of these ants. In this connection the relative amount of food stored, in the tissues of the fat body for instance, per

individual body weight of the different insects has not been investigated, although it must be mentioned as a contributing factor.

This analysis shows that in *Formica ulkei* reduced metabolic activity occurs in both hibernation and starvation. In the former case, however, activity and metabolism are uniformly lowered from the beginning, whereas in starvation the animal remains active, depleting its reserves, and the metabolic rate progressively declines as this occurs. The ultimate result is a greater depletion than occurs in hibernation, and finally death. Fink (1925*a*) expressed the same opinion in respect to his data on the potato beetle undergoing starvation.

DISCUSSION

In the introduction, reference was made to preliminary studies by Holmquist (1928) on the respiration of *Formica ulkei* which was taken as a starting-point for the present work. Certain differences have appeared which should be mentioned. The volume of gaseous exchange reported by Holmquist is extremely low, about 138 cu. mm. of CO_2 per gram per hour at 22° C. Reference to Table III and the work of other investigators mentioned shows that such a volume is too low to be comparable to that obtained on any of the different insects studied. Two factors may be involved here. In the first place, the mercury manometer used by Holmquist is a much less sensitive instrument than the respirometer employed in these experiments. Secondly, he used a large number (100) of ants in an experiment which extended over about 24 hours. It has been pointed out above that the conditions of these experiments, 25 animals and 105 minutes, were found to be optimum for the ants. It is highly probable, then, that overcrowding of the ants may be the explanation for the very low volume of gaseous exchange recorded by Holmquist. The effect of crowding and of isolation upon insect respiration deserves more attention than it has received to date.

The R. Q. for the ants at low temperatures (3°–6° C.) was reported by Holmquist to be higher, slightly above 1.0, than that of the active ants for which he found a mean R. Q. of .888. Such an R. Q. has not been observed for any of the insects on which respiratory investigations have been reported, nor is such an R. Q. expected to result from the alterations in metabolic activity produced by low tempera-

ture. Athanasiu (1909) reported that during hibernation, the R. Q. of frogs is raised from the summer level of .77 to .97, or even to above 1.0. To explain this high quotient, he postulated that a storage of oxygen occurred in the tissues in preparation for hibernation. The use of this stored oxygen during hibernation then accounted for the small consumption observed. Iizuka (1926) did not confirm this observation for frogs during hibernation, but he reported an increase in the R. Q. up to 1.0 or even above when frogs were desiccated for 24 hours. Neither of these interpretations of an increase in R. Q. seems applicable to the present situation, as will be pointed out below. However, the high R. Q. may result from an error introduced into the calculations if formic acid fumes from the ants are absorbed by the KOH along with the CO_2. Furthermore, asphyxiation of the ants results from exposure to confined formic acid fumes. Vernon (1896) observed that asphyxia of a number of marine invertebrates is associated with an increase in the R. Q. to well above 1.0, that is, they continue to produce CO_2 when little or no O_2 is being consumed. Either or both of these conditions may be a factor in the high R. Q. which Holmquist observed for the ants in hibernation, inasmuch as the large number he used would be difficult to handle without considerable disturbance, which always produces an exudation of formic acid from the ants.

It has been pointed out that a decrease from a high rate of respiration, 735 cu. mm. of CO_2 per gram per hour, with a mean R. Q. of .866, to a low rate, 155 cu. mm. of CO_2 per gram per hour, with a mean R. Q. of .500 during hibernation, is characteristic of *Formica ulkei*. Such reactions are likewise typical of numerous other insects for which hibernation has been investigated, and similar to the effect of hibernation on mammals and numerous other animals. The small volume of gaseous exchange is direct evidence of the low rate of metabolism of the hibernating animals. But what factors are involved in this reduced rate of metabolism to produce the low R. Q. which accompanies it?

Many theories, reviewed by Rasmussen (1916), have been advanced to explain the phenomenon of hibernation; but only a few offer any real physiological explanation for the effects that are so uniformly observed, particularly the low R. Q. Two of the hypothe-

ses advanced in this connection postulate a storage of gases in the blood. Regnault and Reiset (1849) were of the opinion that O_2 is stored in the tissues during hibernation, so that the amount of oxygen intake becomes relatively greater than that actually used, and hence considerably greater than the CO_2 produced. On the other hand, Du Bois (1896) presented the theory of carbon dioxide narcosis as a cause for hibernation. This implies an accumulation of CO_2 in the tissues, so that the amount measured is less than that actually produced in the oxidative processes. In view of the volumes of gases that would necessarily be stored in order to account for the low R. Q. during hibernation, neither of these theories seems very plausible; and other inconsistencies in them have been pointed out by many investigators (Krogh, 1916; and Nagai, 1909).

Another cause of the low R. Q. described by Du Bois (1896), Weinland and Riehl (1907), and Frew (1929) is the conversion of fat into glycogen during hibernation, which produces an R. Q. of .700 or less, with the subsequent consumption of the glycogen to account for the R. Q. of about 1.0 which they observed upon the exit from hibernation. This theory is generally criticized on the ground that the amount of glycogen that would be stored is far in excess of that found in the animal body. It would seem to apply to insects only in cases where hibernation occurs during the larval period, for it is then that glycogen is found to increase in the insect body to be used subsequently during pupation (Uvarov, 1929). Only traces of glycogen are found in the adult insect, so that the theory does not seem tenable for insects such as the ants that hibernate as adults. Parhon (1909) found only .14–.21 per cent by weight of glycogen in bees. Further, Timon-David (1928), pointed out that in the hibernation of a butterfly, in which there is an extreme reduction of metabolism, the enormous fat reserve remains constant. It is used up in the transformation into the adult but not as reserve food material during hibernation.

Fink (1925*a*) found that reduced respiratory metabolism is associated with reduced catalase activity and catalase content of the hibernating potato beetle. Bodine (1921*b*) also found that a decrease in CO_2 output normally accompanied decrease in catalase in the grasshopper, firefly, and potato beetle, but that during hibernation

the marked decrease in CO_2 production was not accompanied by the reduction of catalase content. No catalase determinations have been made for the ants.

One of the most commonly observed physiologic changes attendant upon hibernation is the reduction in body water content. Numerous investigators, including Tower (1917), Bodine (1923), Caldwell (1925), and Townsend (1926), have reported a decrease in water content of various hibernating insects of from 15 to 55 per cent of the body weight. It was pointed out by Caldwell that such reduction of water content may affect the metabolic processes through accumulation of waste products, through inadequate distribution of oxygen or food materials, or by direct action in slowing down the oxidative processes. Thus it may well be a causal factor for the low R. Q. observed in hibernation accompanied by a decrease in body water content.

However, the reduction in body water content occurring in *Formica ulkei* during hibernation is considerably less than that of other insects. Holmquist (1928) reported a decrease in hibernating workers of about 5–15 per cent, while a variation of about 5 per cent was observed in the determinations of the water content of the active workers. Evidently, the reduction of water content is not so significant a factor in this case as it may be in some of the other insects. Furthermore, this dehydration appears to be associated with cold hardiness. Bodine (1923), working with two species of grasshoppers, found that *Chortaphaga australior*, which does not normally hibernate, will hibernate like *C. viridifasciata* when exposed to the same conditions, and that the physiological reactions of *C. australior* are then the same, including reduction in water content, as those of *C. viridifasciata* during hibernation. Tower (1917) observed that potato beetles, when raised in a warm desert region, do not undergo the reduction of water content during hibernation which accompanies their hibernation under conditions of low temperature. Payne (1926*a* and 1928) showed that dehydration is associated with cold hardiness of the Japanese beetle larvae and several oak borers to both the quantity and intensity factor of low temperature. In addition it is known (Babcock, 1912; and F. G. Hall, 1922) that water produced in metabolic processes, i.e., metabolic water, is an important

source of the body water content of many insects. Since the rate of metabolism is greatly reduced at low temperature, it is obvious that a concomitant reduction in metabolic water and hence in body water content would occur. Evidently, if reduction of water content is a factor in the alteration of metabolic processes and the lowering of the R. Q. during hibernation, it is intimately associated with another factor, namely, temperature.

The direct correlation of the volume of gaseous exchange and the magnitude of R. Q. to temperature which has been shown for *Formica ulkei* indicates that it is an important factor affecting the metabolic processes of the insect. In fact, the respiratory exchange and the R. Q. observed appear to be direct indicators of the environmental temperature through its effect on metabolism. Reference has already been made to a similar opinion expressed by Kayser and Ginglinger (1927) for non-hibernating mammals. They believe that the observed R. Q. represents the algebraic sum of the R. Q. of each of several oxidative processes going on at any one time, the equilibrium of which varies with temperature.

Nagai (1909), studying the metabolism of several hibernating mammals, expressed a similar opinion in a slightly different manner. He believes that oxidation intensity during hibernation varies with the body temperature and that the lowering of the R. Q. is due to imperfect and incomplete oxidative reactions caused by the lowered body temperature, and not to conversion of fat into sugar or to gas storage in the tissues. Such abnormal metabolism he found to be further substantiated by the great displacement of nitrogen distribution and the presence of lactic acid in the urine. Such a concept seems applicable to the data at hand not only in respect to the effect of temperature but also in respect to that of starvation. It has been shown that the rate of respiration and the R. Q. of *Formica ulkei* vary in respect to diet in the manner expected from the known effects of diet upon metabolic processes and that a great reduction in gaseous exchange and R. Q. is associated with starvation. However, starvation cannot be interpreted as a causal factor for the low R. Q. observed in hibernation, since it has also been shown that the inactivity and altered metabolism associated with low temperature protect these ants from starvation during hibernation. From this anal-

ysis of the physiological effects of hibernation upon *F. ulkei*, it appears that temperature is the major factor concerned in the metabolic changes which occur.

In conjunction with this discussion of the physiological effects of temperature upon these ants, it is interesting to point out how their behavior is modified in nature in response to temperature as an environmental factor. Baumberger (1914) observed that, while various factors are involved in insect hibernation, the dominant factor in any one case is the one to which the insect is most sensitive or which is nearest its vital limits. Now, it is generally admitted that not all insects hibernate in response to low temperature, as Baumberger (1917) has shown specifically for the codling moth. Some beetles begin to hibernate before low temperature sets in (Kirby and Spence, 1817–63). Others hibernate periodically as a necessary part of the life-cycle. The rest period or exposure to cold of hibernation is often essential to emergence from pupation or to further reproduction. However, many insects, such as *Drosophila*, the house fly, and the ants, hibernate in response to temperature changes. Roubaud (1925) calls such insects, which exhibit no obligatory hibernation but which show a direct correlation in activity to temperature, "homodynamic insects."

Some authors imply that the hibernation of such homodynamic forms is merely cold torpor and not true hibernation. However, if metabolic changes are a criterion of true hibernation (and it is generally believed that they are), then *Formica ulkei* is a homodynamic insect which undergoes a true hibernation and not merely cold torpor in response to low temperature. According to Baumberger, this is the factor to which the ants are most sensitive. Such an idea is compatible with ant behavior in general, as the following instances will illustrate.

The eggs, larvae, and pupae of ants are constantly moved around in the nest by the workers in response to optimum conditions of moisture and temperature. Cobelli (1903) observed that several species of ants enter upon and exit from hibernation in direct correlation to their sensitivity to cold. Miller and Gans (1925) experimentally caused ants to change their path, first positively and then negatively, in response to radiant heat of varying intensity. Steiner (1924) and

Andrews (1927) have shown that regulation of temperature occurs in ant mounds. According to Steiner, this temperature control is due to respiratory heat produced by the ants and is associated with the social life, as in the case of over-wintering bees. He terms it "social heat regulation" and indicates that it implies not only a well-developed individual temperature sense but also a social heat instinct.

With such evidence of the relation of ants to temperature in mind along with the physiological data showing the metabolic reactions to temperature, it is easy to accept the temperature mechanism which Holmquist (1928) postulated for the entrance to and the emergence from hibernation of *Formica ulkei*. The description of this process, confirmed by present observations, is as follows: As temperature declines in the fall, the lowering of the air temperature causes a similar change in the surface soil, but the deeper parts of the nest are only slightly affected. The result is a reversal of the temperature conditions in the mound, so that the lowest temperatures occur at the surface and progressively higher temperatures are found toward the soil-water level. In order to escape the low temperatures in the upper parts of the nest, the ants retire as far as possible from the colder surface soil to the warmer levels below. The arrival of ants from above and the upward migration of those from below to escape the rising water level results in the crowding of the ants into the densely packed masses typically found in the lower galleries during hibernation. Emergence in the spring is likewise a temperature phenomenon. This is shown by the fact that surface activity begins as soon as frost is out of the ground and gradually increases in intensity until all ants have left their hibernation quarters and become active at the surface. A few scattered ants that overwinter in the frozen soil of the mound are roused to activity first when the surface soil becomes warmed in the spring. In some nests, at least, the hibernating masses of ants are forced upward at about the same time by the encroaching soil water. This not only brings them nearer the warmer surface but places them within reach of the already active surface ants. It is possible that the latter may drag individuals from the hibernating masses toward the warmer surface, thus aiding and hastening the exit from hibernation.

It is evident that these references to the bionomics of ants support the experimental findings, so that we now have a functional picture of the seasonal reactions of these ants to temperature based upon the observed metabolic changes and their natural responsiveness to temperature as an environmental factor. Let us summarize the argument in a few concluding statements: First, the data show that the intensity and character of the respiratory exchange of *Formica ulkei* are fundamentally modified by changes in temperature. Second, reduction in body water content is not as significant a change accompanying the hibernation of *F. ulkei* as it is in the case of many other insects; under any circumstances, dehydration seems to be associated with cold hardiness, and consequently its physiological effects are closely tied up with those of temperature. Third, although starvation is a factor capable of producing such abnormal metabolic processes as are indicated by a low R. Q., it has been shown that hibernation of *F. ulkei* normally protects the species from starvation. Fourth, other factors which, under certain circumstances, may be associated with reduced respiratory exchange or low R. Q. are not applicable to these data. Fifth, temperature is a factor to which these ants are very responsive in nature and which appears to be sufficient as a mechanism to account for their normal behavior. Consequently, temperature must be recognized as the primary causal factor involved in the hibernation of *F. ulkei*.

CONCLUSIONS

1. The normal respiratory exchange of *Formica ulkei* is determined and is found to exhibit a seasonal rhythm.

2. Ants living under conditions of constant temperature maintain a constant rate of respiration and a uniform R. Q. throughout the four seasons.

3. The rate of respiration and the R. Q. vary directly with the experimental or environmental temperature.

4. There is a reduction of 79 per cent in CO_2 production and 63 per cent in O_2 consumption between the active ants at 22° C. and the hibernating ants at 4° C.

5. The mean R. Q. for active and hibernating ants is .866 and .500, respectively.

6. The R. Q. varies according to the limitation of the diet. It is .984 for carbohydrate, .711 for protein, and .634 for fat.

7. Starvation produces a marked reduction in the volume of respiratory exchange and in the R. Q. It is more pronounced than that produced by hibernation, i.e., 92 per cent for CO_2 and 85 per cent for O_2.

8. Normally, hibernation protects the ants from starvation during their period of inactivity at low temperatures.

9. The analogous effects of low temperature and starvation on the rate of respiratory exchange and the R. Q. of *Formica ulkei* and the inadequacy of other physiological explanations for such changes in the R. Q. indicate that abnormal and incomplete oxidations due to low temperature are the cause for the reduced metabolism occurring during hibernation.

10. Temperature is described as the primary factor involved in the hibernation of *Formica ulkei* which is shown to be a type of social homodynamic insect.

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